

Exobiology Branch Overview

Research in the Exobiology Branch focuses on the advancement of the scientific understanding of the origin and distribution of life by conducting research on the cosmic history of biogenic compounds, prebiotic evolution, and the early evolution of life. This is accomplished via laboratory experiments, theoretical studies/computational modeling, and field investigations. Branch personnel are also involved in the development of flight instruments, experiments, and small mission definition with particular emphasis being placed on studies of Mars and the development of instrumentation for martian flight missions. Several Branch scientists are part of a task module that is part of the Ames membership in the Astrobiology Institute. Branch scientists provide expertise in exobiology, astrobiology, planetary protection, and other areas of planetary science to NASA Headquarters and external review and advisory panels, and some serve as editors and associate editors of scientific journals.

M. Fonda, Chief SSX



The Ecology of Modern Microbial Mats and Stromatolites

Brad Bebout, Pieter T. Visscher, and Jack Farmer

Work in the Microbial Ecology/Biogeochemistry Research Lab focuses on characterizing the biological activity of microbial mats and stromatolites. This is accomplished using a combination of field and laboratory studies of modern microbial mat and stromatolite communities. Microbial mats (laminated microbial ecosystems occurring in a variety of aquatic environments) and stromatolites (which can be thought of as mats in which minerals are being trapped and/or precipitated) are modern examples of the most ancient biological communities on Earth, examples of which are preserved in rocks as old as 3.5 billion years.

Mineral formation in microbial mats, which occurs to make stromatolites, is important to understand as it is primarily the stromatolites, and not the microbial mats, which become a part of the fossil record of life on Earth. Particular key questions being addressed are: What are the conditions under which these mat communities actually 'turn to stone'? What happens to the organisms building the community as this happens? What are the biological consequences? In collaboration with a team of scientists led by R. Pamela Reid (University of Miami) and funded by the National Science Foundation, studies of stromatolites found in the Bahamas have resulted in a model of one set of processes leading to lithification (formation of rock).

Trace gas production and consumption in microbial mats is important to understand for a number of reasons. In addition to representing a potential loss of organic matter by the community, these gases diffuse into the atmosphere, where they can affect climate (as in the case of dimethyl sulfide and methane). In addition, the detection of some of these gases in the spectra of atmospheres of extrasolar planets is considered to be a viable search strategy for the detection of life on extrasolar planets. Biological processes within these communities control the amounts and timing of the release of various trace gases. The trace gases produced and consumed by microbial mats from various environments have been quantified, and some of the factors affecting these rates of production consumption have been identified.

Technology development in the lab has centered on finding better ways of measuring rates of metabolic processes, the concentrations of biologically important chemical species, and the rates of production and consumption of trace gases in situ (in the natural environment). Naturally occurring microbial mats and stromatolites are subjected to conditions of water flow, temperature, and sunlight which are difficult, if not impossible, to simulate in the laboratory. Extensive fieldwork in Yellowstone National Park, the Bahamas, and Baja California, Mexico has therefore been undertaken. Preliminary versions of a

device designed to make in situ measurements of oxygen concentrations within these communities at 100-micrometer spatial resolution were tested and evaluated. New types of sensors designed to measure the light available for photosynthesis within these communities are also under development. A new device designed to measure the degree

of lithification (as hardness) within stromatolites was developed and tested in the Bahamas. □

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Evaluation of Evidence of Life in the Martian Meteorite ALH84001

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In 1996, an announcement was made that fossil evidence of bacterial life had been found in a meteorite known to have originated from Mars. One of the main lines of evidence for the putative bacterial fossils in the Martian meteorite ALH 84001 is the presence of submicroscopic crystals of the mineral magnetite that are similar in size and shape to biogenic magnetite crystals formed by primitive bacteria on Earth. The terrestrial organisms, called magnetotactic bacteria, use the magnetite to navigate in the Earth's magnetic field. While some bacterial species are able to mediate the deposition of minerals in the local extracellular environment, magnetite from terrestrial magnetotactic bacteria is known to be formed only within the cell membrane of the bacterium. In ALH84001, the submicroscopic magnetite is found in large aggregations associated with iron- and magnesium-rich carbonates, and the supposition is made that the magnetite

survived the demise of the organisms and was passively incorporated into the surrounding carbonate. In this report, new evidence is presented concerning the ALH84001 magnetite which points to an inorganic origin.

This work utilized a petrographic thin section of the meteorite, obtained from the curatorial collection at Johnson Space Center. Small portions of the meteorite material which contained both carbonate and magnetite were removed from the thin section and attached to electron microscope sample grids. The grids were placed in a sample preparation device which milled the material to a thickness less than 0.1 μm (one ten millionth of a meter, or about 1/1,000 the diameter of a human hair). The thinned material was viewed in a high resolution Field Emission Transmission Electron Microscope (FETEM) at the



National Center for Electron Microscopy, Lawrence Berkeley Laboratories. In the FETEM, magnifications are achieved in which planes of atoms making up the magnetite and carbonate crystal structures can be directly viewed.

At high magnifications, the relationship of the magnetite crystal structure to that of the carbonate crystal structure is apparent. Figures 10a-b, for example, show a high-resolution image of a single crystal of magnetite within the carbonate matrix. The fine lines in 10b are

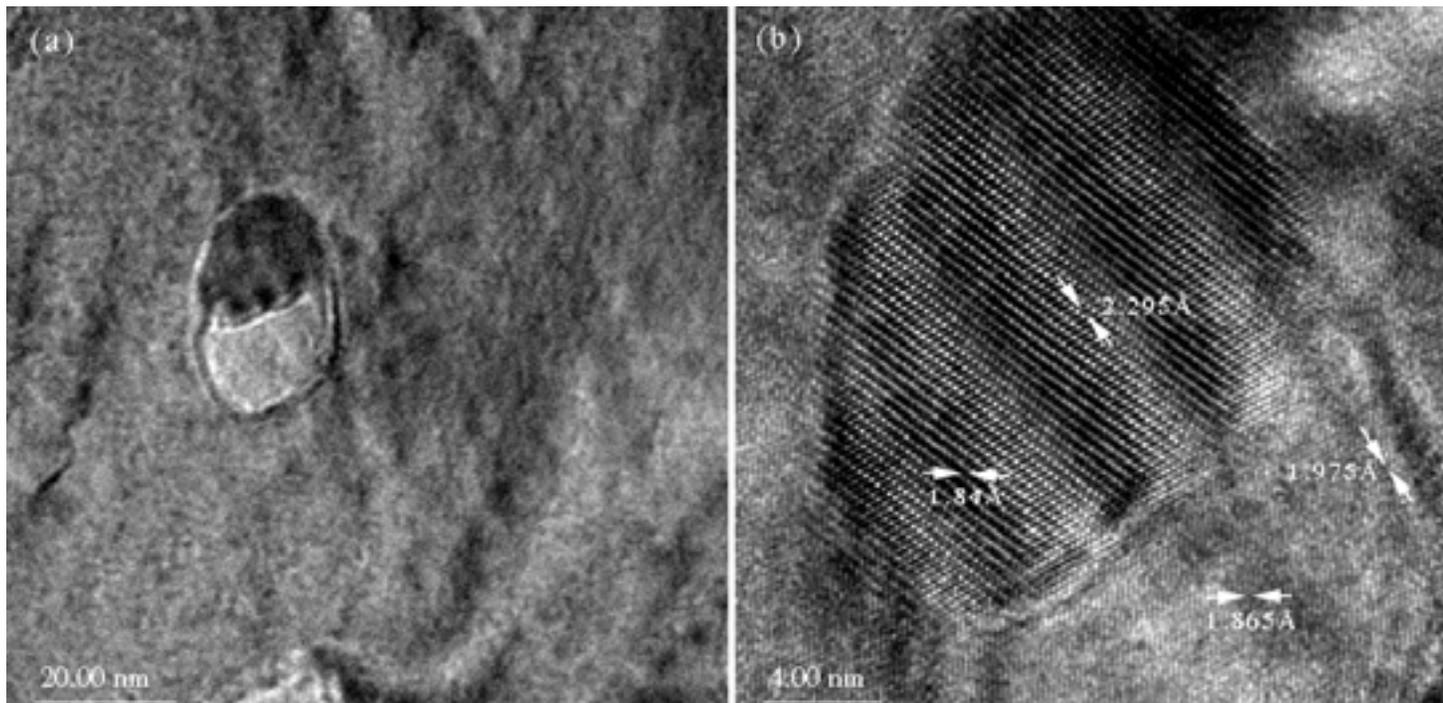


Figure 10. High-magnification images of magnetite in epitaxial orientation to enclosing carbonate. (a) view of a single magnetite crystal enclosed within a small hole in the carbonate. (b) Lattice-resolution image of the magnetite and carbonate shown in (a). Atomic planes in the magnetite have closely similar spacing and parallel orientations to those in the carbonate, typical of an epitaxial relationship. The likelihood that this orientation occurred randomly after the magnetite was formed (e.g., within a bacterial cell) cannot be entertained.

planes of atoms making up the crystal structures of the carbonate and magnetite phases. One can see that certain planes of atoms or crystallographic directions within the magnetite structure are in alignment and have spacing similar to planes of atoms, or crystallographic directions of the surrounding carbonate. A significant number of magnetites was found to be related to the surrounding carbonate in this way. This preferred orientation, which is called epitaxy, is proof that the magnetite nucleated and grew on the surrounding carbonate. If the magnetite nucleated and grew at a site removed from direct contact with the carbonate (for example, within the membrane-bound body of a bacterial cell) and was later deposited in the carbonate, it would be highly improbable that such a special orientational relationship would arise.

Not all magnetite exhibits an epitaxial relationship with the surrounding carbonate within ALH84001. However, it is highly unlikely that magnetite crystals which do not exhibit epitaxy but which occur in identical spatial and temporal settings within the meteorite, were formed by an entirely different mechanism. Indeed, evidence obtained by Ames researchers suggests that the magnetite was precipitated inorganically from a carbonate-rich hydrothermal solution. The presence of nearly identical spacing between atomic planes in the carbonate and magnetite phases facilitates nucleation of the magnetite onto the carbonate from sparingly saturated hydrothermal solutions. Epitaxial growth of one phase on another avoids the activation energy barrier associated with homogeneous nucleation, a significant hin-

drance to crystallization in most systems. During continued crystallization, a level of supersaturation must have been achieved in which magnetite could precipitate directly from solution without the requirement for a precursor phase.

Evidence now points to an inorganic origin for many of the features reported from the ALH84001 meteorite. However, analyses of the meteorite do suggest that carbonate-rich hydrothermal solutions existed in the near-surface environment on Mars 3.9 billion years ago, around the time that the first evidence of life is found on the Earth. It remains that Mars is one of the most interesting objects in the solar system from an Astrobiological point of view, and evidence of hydrothermal systems and carbonate deposition in the early wet and warm period of Mars history only increases our interest in that planet. □

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Progress on a Laboratory Version of CHEMIN, An X-Ray Diffraction X-Ray Fluorescence Instrument

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CHEMIN is a miniaturized Charge Coupled Device-based (CCD) X-ray diffraction/X-ray fluorescence (XRD/XRF) instrument. The name CHEMIN refers to the instrument's combined CHEMical and MINeralogic analytical capability. The instrument, for which a patent was awarded to NASA in 1996 ("X-ray diffraction apparatus," US Pat. No. 5,491,738) is designed to characterize the major element composition and mineralogy of small fine-grained or powdered samples. Both diffraction and fluorescence data are obtained simultaneously by exposing a 2-dimensional CCD imager directly to the X-rays and sorting out the photons according to their energies. CHEMIN was originally designed as a spacecraft instrument, and has been proposed for five flight missions including most recently the Mars 2001 opportunity. However, during the development and characterization of the flight instrument, it was found that CHEMIN was superior in many ways to the laboratory instruments from which it was derived. The CHEMIN design team has recently begun adapting the flight instrument for use in terrestrial laboratories and in remote locations on the Earth.

There are several aspects of the design that make the instrument ideal for certain types of laboratory analysis. CHEMIN is the first combined

XRD/XRF device for which data of both types are recorded concurrently from the same micro-area of the sample. Areas as small as 30 micrometers in diameter can be analyzed using conventional laboratory X-ray sources. The CCD detector is 2-dimensional, allowing for detection of the entire front reflection region of the diffraction pattern, from the primary beam direction to nearly 60 degrees in 2-theta space. This results in a nearly 2-order of magnitude improvement in counting rate over conventional X-ray diffractometers. In addition, because full debye rings are collected, one can record fully quantitative data from as-received or poorly prepared materials. Preferred crystal orientation and variation in crystallite size, important parameters in geology, biology, and materials science can be assessed directly using CHEMIN.

Because the CCD detector can discriminate X-ray photons according to their energies, several diffraction patterns can be collected at once, using different X-ray source energies. This allows concurrent collection of data from large 'd-value' materials such as clays at the same time as small d-value materials such as ceramics. The real-time nature of data collection will allow researchers to follow the course of reactions or phase changes using X-rays in much the same way that

Fourier Transform Infrared Spectroscopy is performed today. The CHEMIN laboratory instrument is equipped with a miniaturized (about the size of a pencil stub) low-power micro-machined field emission X-ray source developed by MOXTEC, Inc. as a Small Business Innovative Research (SBIR) project. An advanced CCD with improved X-ray energy resolution, developed by Jet Propulsion

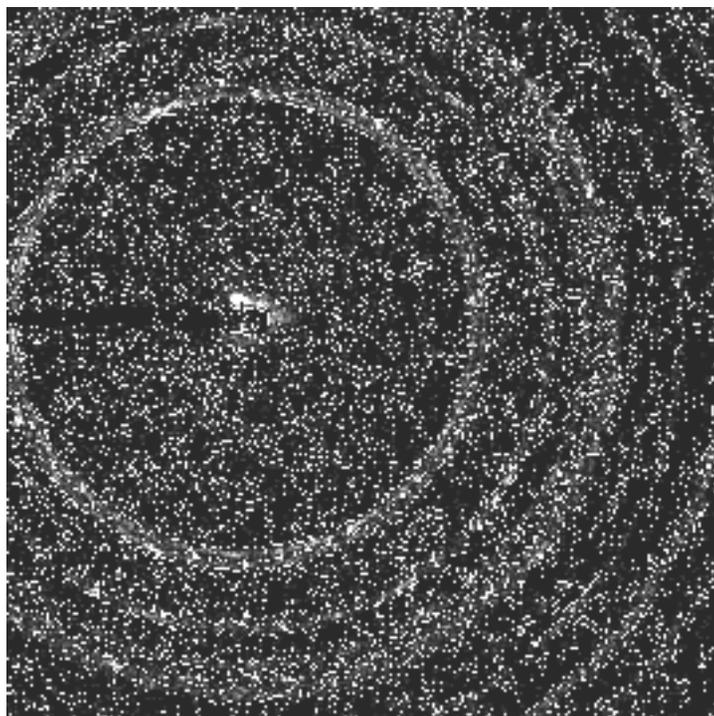


Figure 11. Diffraction pattern for Aragonite, CaCO_3 using an unfiltered copper X-ray source. The pattern is comprised of monochromatic copper K photons, chosen according to their energy.

Laboratories for an astrophysics mission will be used as a detector for the instrument. X-ray diffraction data are recorded (in transmission mode), for atomic spacings from 1.66 to 15 Ångstroms, a range which covers definitive maxima for nearly all minerals (see, for example figures 11 and 12).

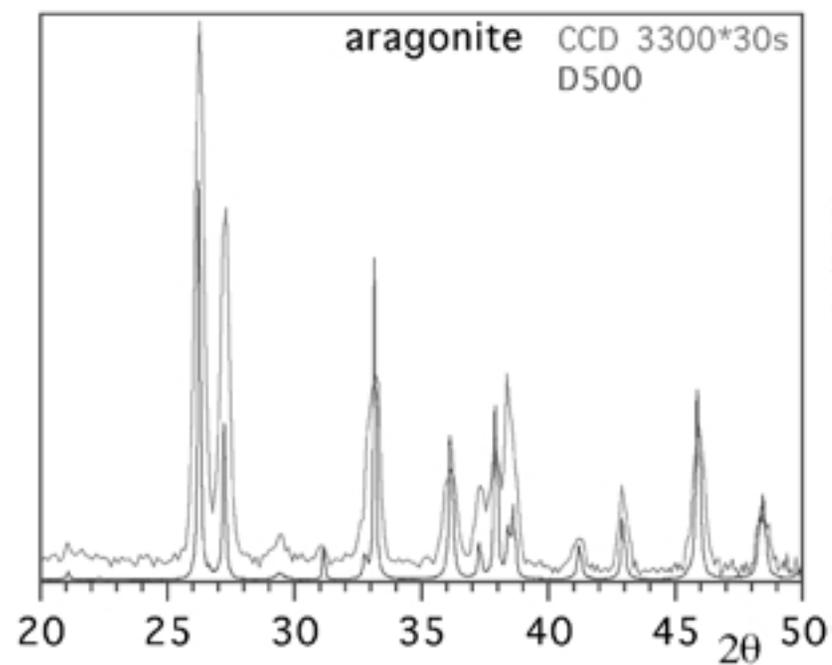


Figure 12. Aragonite diffractogram, assembled from the data of Figure 11. The inset pattern, also aragonite, was obtained using a commercial laboratory instrument. The peak width of the CHEMIN instrument is 0.2 degrees 2 theta, as compared to 0.06 degrees 2 theta for the commercial instrument. Despite the slightly greater peak width, CHEMIN data are fully quantifiable using Rietveld refinement.



X-ray fluorescence data may be obtained for elements $4 < Z < 92$ (beryllium to uranium) (see figure 13). Energy discrimination is used to differentiate between diffracted primary beam characteristic photons and secondary fluorescence photons. The diffraction mode line resolution of 0.02 \AA is sufficient to allow application of the Rietveld refinement method to the data. The proposed CHEMIN development effort

will include combining diffraction and fluorescence data using simultaneous linear equations, to derive the most accurate mineral and chemical composition. A prototype version of the CHEMIN instrument, developed with NASA Planetary Instrument Definition and Development Program funding has been operable since July, 1996. □

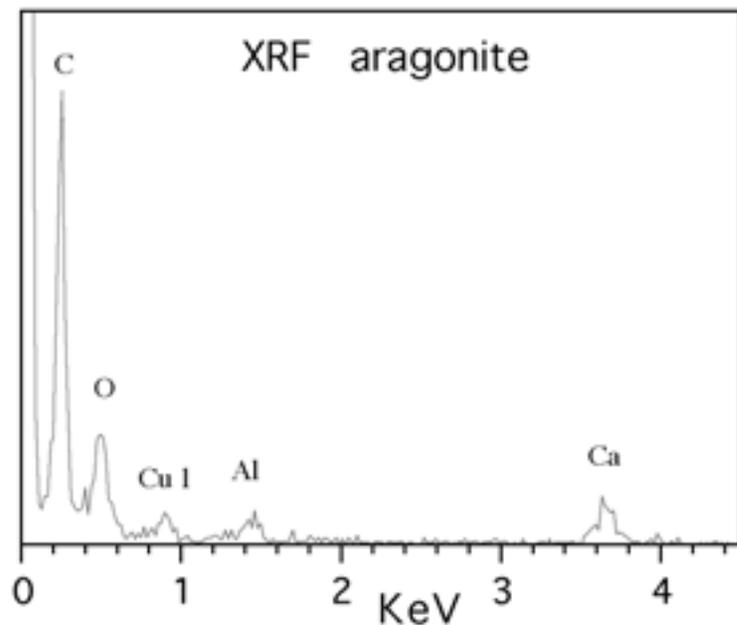


Figure 13. Aragonite XRF spectrum. Aluminum and copper X-rays originate from components of the camera system of the prototype instrument and are not part of the sample.

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Polyhydroxylated Compounds in Meteorites

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Meteorites and interplanetary dust particles are the oldest items available for laboratory study of early solar system chemical and physical processes. Most carbonaceous meteorites, a class of meteorite relatively enriched in carbon and organic compounds, are as old or older than the solar system planets, approximately four and a half-billion years. As a group they are also the most unaltered early solar system objects as illustrated by their near solar (Sun) abundances in all but the most volatile elements. For this reason their content of organic compounds is a record of the earliest known abiotic organic synthesis. Meteorites, along with comets and interplanetary dust particles, may have delivered much organic matter to the early Earth.

The best characterized carbonaceous meteorite with respect to organic chemistry, the Murchison meteorite, has been found to contain a large number of water-soluble organic compounds. These include amino acids, carboxylic acids, dicarboxylic acids, hydroxycarboxylic acids, amides, purines, pyrimidines, phosphonates, and sulfonates. Absent among the biologically important compounds reported in meteorites are sugars, polyhydroxylated aldehydes or ketones (polyols). Ribose and deoxyribose, five carbon sugars, are central to the role of contemporary nucleic acids, DNA and RNA. If polyhydroxylated compounds are found in meteorites, this would demonstrate that such compounds could have been part of the initial mixture of prebiotic and biologically

important compounds on the early Earth. If polyols, or any series of organic compounds are true products of abiotic chemistry, it seems likely that their synthesis would begin with smaller members of the series and gradually build, in decreasing abundance, to more complex members. This is the case with all homologous series of indigenous organic compounds seen in the Murchison meteorite, i.e., amino acids, carboxylic acids, amides, etc. In the case of sugars and other polyols, one of the most generally agreed upon scenarios for natural abiotic synthesis is the 'Formose' reaction. In this reaction, formaldehyde (CH_2O) in aqueous solution reacts with itself to gradually build a variety of hydroxylated compounds and sugars of increasing carbon number. Among the known products are glycoaldehyde, ethylene glycol, glyceraldehyde, dihydroxyacetone, glycerol, erythrose, ribose, six carbon sugars, etc. Because there was aqueous alteration on the parent bodies of carbonaceous meteorites and formaldehyde is a ubiquitous interstellar compound, the Formose reaction would have been possible in meteorites. Efforts at Ames have been directed towards determining the nature and abundance of polyols in carbonaceous meteorites.

The methods used for the study include isolating the meteorite polyols by ion exchange chromatography, identification by gas chromatography-mass spectrometry (GC-MS) and isotopic analysis to



ensure that identified compounds are extraterrestrial and not Earthly contaminants. Preliminary analysis of Murchison extracts indicates an abiotic synthesis of polyols, i.e., a series of compounds of increasing carbon number (and decreasing abundance) have been observed. Some of the identified compounds are ethylene glycol, glycerol, dihydroxyacetone and glyceric acid. There is also evidence of higher polyols. Bulk carbon ($^{13}\text{C}/^{12}\text{C}$) and hydrogen (D/H) isotopic measurements also indicate that the majority of these compounds are indigenous to

the meteorite. Determination of the isotopic composition (D/H, $^{13}\text{C}/^{12}\text{C}$, and $^{16}\text{O}/^{17}\text{O}/^{18}\text{O}$) of individual compounds as well as further bulk measurements will help to determine their synthetic origins. □

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Evolutionary Ecology of Microbial Communities in Yellowstone

Ken Cullings

Ecosystem function and responses of ecosystems to both human-induced and natural change are mediated to a large extent by microbial processes. While this fact is widely acknowledged, the role that different microbes play in this mediation is poorly understood. This is because the microbial community can be likened to a 'black box,' it is extremely difficult to identify microbes in their natural habitat because there are few if any physical characteristics that can be used to distinguish among them. In this research, molecular-genetic tools are being developed for microbe identification in soil samples. These tools are being used to answer fundamental questions regarding ecosystem function and microbial evolution.

One set of projects is aimed at asking to what degree co-evolution has given rise to specific interactions in a special type of plant/microbe interaction, the mycorrhizal symbiosis. Mycorrhizae are composed of plant and fungal components, and are responsible for mediating photosynthesis, carbon cycling, soil litter breakdown, and nitrogen cycling in all terrestrial ecosystems. If patterns of specificity exists in nature, and if given plant species therefore require a certain subset of fungal species to survive, then the presence or absence of given fungal species would affect ecosystem dynamics. In addition, the question of whether or not human-induced disturbance to ecosystems, such as clear-cutting, and natural disturbance, such as fire, affect mycorrhizal communities differently is being addressed.

Finally, because mycorrhizae enable plants to establish and survive in extreme environments, how mycorrhizal communities in undisturbed ecosystems and thermally-altered soils differ, and what adaptations these microbes have evolved in order to survive in these harsh environments, are being determined.

This research is taking place in the forest and hot spring ecosystems of Yellowstone Park, and is directly addressing many of these questions in the context of the 1988 fires. Results of the co-evolution experiments indicate that specific patterns of interaction have evolved between plant and fungal partners. This indicates that exclusive patterns of carbon and nitrogen flow exist in this forest ecosystem, and suggest that mycorrhizal interactions play important roles in controlling ecosystem dynamics. Furthermore, plants that arrive in the ecosystem relatively late prefer fungal species that break down the needles of the plants that precede them. Therefore, there is evidence that fungal species facilitate the establishment of new tree species, providing rare support for a facilitation model of ecosystem dynamics. This research is being expanded to determine effects of alteration of carbon flow to the roots and of litter manipulation on specificity patterns.

A parallel project addresses the hypothesis that natural burn and clear-cutting will have different effects on the mycorrhizal community. The dogma is that they will not. However there are obvious differences between fire and clear-cutting including super-heating of soils and removal of litter, an important substrate for fungi and the main source

of nitrogen for trees. Initial experiments indicate that there are differences between fire and clear-cutting in the short term. Experiments are now being planned to determine whether or not these differences extend into relatively long time scales, and therefore whether or not human-induced disturbances will have long-term effects on ecosystem dynamics.

Finally, a new project has been started to determine the effects of thermal-alteration of soils on soil microbial communities. This work has direct application to Astrobiology and Exobiology in that the earliest terrestrial organisms, and possibly organisms on Mars, first evolved in hot springs environments. Migration into the surrounding soils would have followed shortly thereafter, and indeed these communities may have persisted long after thermal features dried and springs disappeared. Thus these soils are suitable models in the search for life on Mars and other terrestrial planets. Initial results indicate that acid-sulfate thermal soils are inhabited by fungi that have adapted to extremely acidic conditions, but are different from those traditionally thought to inhabit extreme acid soils. This has important implications for soil remediation strategies, and indicate that the dominant paradigm for fungal adaptation to extreme environments needs modification. This study is being expanded to include other soil microbes such as bacteria, Archea, and cyanobacteria. □

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Peroxy in Rocks - A Paradox

Friedemann Freund and Andrew G. Tenn

The Earth – like any other solar system body, small and large – is chemically reduced. As a result, all igneous rocks from deep within the Earth are reduced. It may therefore come as a surprise to learn that such rocks are suspected to contain peroxy, the epitome of oxidizing compounds. An experimental verification would be quite important and may affect our thinking about rocks, about some of their key geophysical and geochemical properties, and even about some puzzling observations from the realm of biology regarding the earliest life forms on Earth.

A peroxy consists of two oxygen anions. Normally, these oxygen anions carry two extra electrons to complete their outer shell and achieve a stable configuration of eight. In the case of peroxy, both oxygens have given away one of their extra electrons, leaving seven; this is not a stable configuration. By pairing up and forming a short bond of shared electrons, the peroxy is stabilized.

To 'see' peroxy in a mineral structure we have to break the oxygen-oxygen bond. When this happens, the peroxy awakens from its dormant, electrically inactive state releasing defect electrons or 'holes.' These are charges not dissimilar to the holes in a p-type semiconductor used for building transistors. As soon as the holes appear, they crowd

to the surface of the molecule, leading to a positive surface charge and to an enhanced surface conductivity.

This led to a benchmark experiment. Two thin strips of gold were deposited on the surface of a single crystal of magnesium oxide to measure the surface conductivity. Figure 14 shows in (a) a view down onto the crystal on which the two thin gold electrodes, and in (b) a cross section of the conductivity cell with the crystal between two planar electrodes for applying a cross field. The breaking of peroxy bonds inside the crystal leads to an increase in the surface current because the holes come to the surface from within. We then apply a strong cross field and alternate the field direction.

Figure 15 shows how the surface current (bold solid line) changes with the application of ± 1250 Volt-per-centimeter cross fields (thin dashed line). When the cross field is positive, meaning that the upper electrode in figure 14 is positively biased, the surface conductivity decreases. When the cross field is negative, the surface conductivity increases. The effect is reversible during repetitive heating and cooling cycles. Such a response is only possible if the surface conductivity is caused by charges coming to the surface from inside the crystal. The only source for such charges are the peroxy in structure.

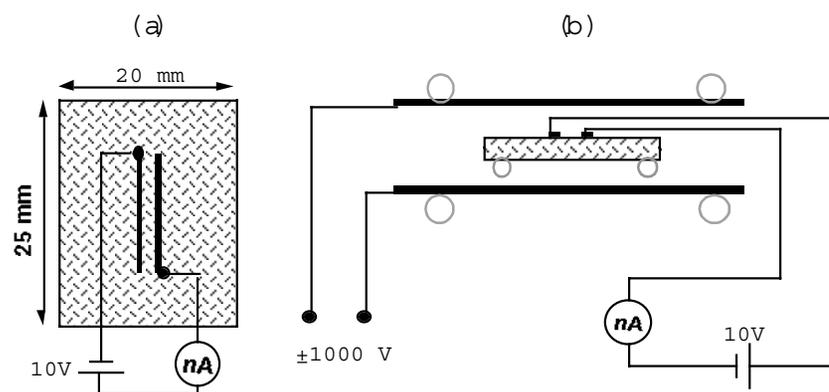


Figure 14. (a) Gold electrodes, 1 x 15 mm, 3 mm apart, sputtered onto one side of a magnesium oxide crystal (patterned) for surface current measurements; (b) Cross section of the conductivity cell with the crystal mounted between two electrodes for application of ± 1250 V/cm cross fields.

Next the surface of the crystal was covered with a very thin layer of carbon and its conductivity re-measured. The conductivity was uniformly higher, indicating that it was dominated by current flowing through the thin carbon film. Application of the ± 1250 V cross fields now ceased to have any effect on the surface current because the electrons flowing through the film are completely separate from the charges that move to and fro inside the crystal.

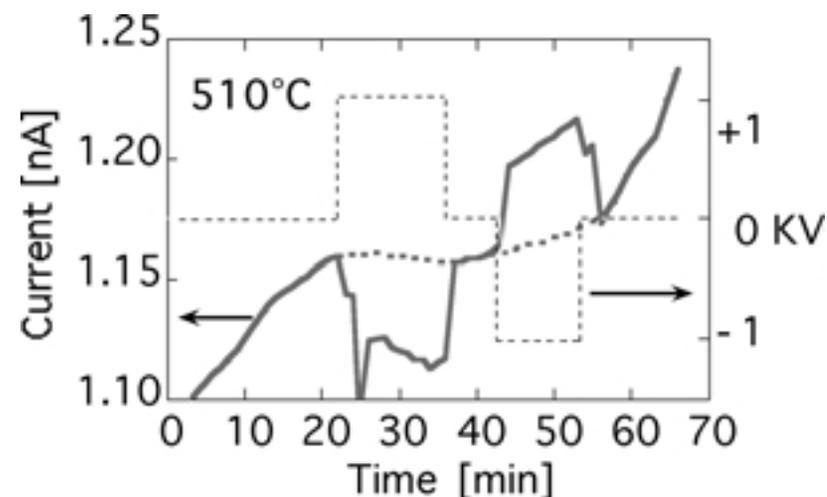


Figure 15. Upon application of +1000 V cross voltage the surface current decreases while at a cross voltage of -1000 V it increases. This is proof positive that the current is carried by positive charges, so-called holes, and that these charges exist inside the magnesium oxide crystal. The baseline drift is caused by slight temperature variations during the run.

This experiment provides proof positive that peroxy exist in a crystal like this magnesium oxide which had been grown from a highly reduced melt and should therefore never contain anything as oxidized as peroxy. The reason why peroxy got into the crystal has to do with the small amount of water that became incorporated when the magnesium oxide crystallized from the melt. During cooling, this dissolved water converted to peroxy and hydrogen. With knowledge available



from other studies it is possible to boldly extrapolate to rocks. All igneous rocks on Earth and Mars have solidified from magmas, generally highly reduced and always saturated with water. Based on these experiments, the minerals in these rocks should therefore contain peroxy.

The presence of peroxy has implications for many geophysical questions related to the electrical conductivity of the Earth's deeper crust and to phenomena associated with earthquakes. Another aspect relates to the earliest life forms on Earth. Rocks that contain peroxy, when they weather, will release hydrogen peroxide. Any early organism living on such a rock surface will therefore experience a constant trickle of hydrogen peroxide, a potentially lethal oxidant. Assuming that early organisms lived in close contact with rocks and secreted acids which etched the rock surface away, these organisms were forced to develop a defense mechanism against this powerful oxidant. It has long puzzled observers that antioxidant enzymes appear deeply rooted on the tree of Life, implying that these enzymes were 'invented' early in life's evolution, long before there was free oxygen gas in the Earth's atmosphere. Peroxy in the rocks and hydrogen peroxide at the rock-water interface may have been the driving force behind this important early evolutionary step. □

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2-Methylhopanids: Biomarkers for Ancient Cyanobacterial Communities

Linda L. Jahnke, Roger E. Summons and Harold P. Klein

A variety of microbially mediated processes contributed to the cycling of carbon in ancient microbial ecosystems. These processes can sometimes be characterized by the identification of remnant cellular compounds ('biomarker molecules') in ancient sedimentary organic matter. One of the important goals of biomarker analysis is to link these ancient chemical fossils to modern day organisms and understand through study of contemporary environments how much biomarkers are deposited, how they survive, and what they tell about the evolution of ancient microbial communities. An important new tool in this work is being applied to biomarker analysis. Compound specific isotope analysis (CSIA) allows measurement of the relative amounts of carbon-12 (^{12}C) and carbon-13 (^{13}C) in any compound which can be chromatographically separated using a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS). Biochemical processes often result in the synthesis of molecules depleted in ^{13}C relative to ^{12}C . Such carbon isotopic fractionations are described by the term 'delta C13' ($\delta^{13}\text{C}$). CSIA allows us to determine $\delta^{13}\text{C}$ values for individual organic compounds. This is important because as carbon is cycled through a microbial community the preference of biological systems for ^{12}C leads to synthesis of lighter biomarker molecules as the carbon flow approaches terminal pro-

cesses, so that an additional degree of taxonomic attribution results in measurement of the $\delta^{13}\text{C}$.

Life began on the early Earth in an environment devoid of free molecular oxygen (O_2) in the atmosphere. The rise of O_2 in the atmosphere, as a consequence of the evolution of oxygenic photosynthesis, had a profound effect on the subsequent progression of the Earth's biological evolution and geological history. Oxygenic photosynthesis evolved within the cyanobacterial lineage, however, evidence for the timing of this event remains obscured within the molecular and rock records. By studying the molecular structure and carbon isotopic composition of lipid biomarkers in cyanobacteria, we seek to link modern day cyanobacteria and their microbial mat analog environments to an ancient organic record.

The bacteriohopanepolyols (BHP) are a group of amphiphilic membrane biochemicals which are a particularly important group of biomarker molecules because the hydrocarbon skeletons of BHP are extremely refractory and survive sedimentary diagenesis. The geohopanes may well be the most abundant class of natural products on the Earth. BHP are widespread among the aerobic bacteria and

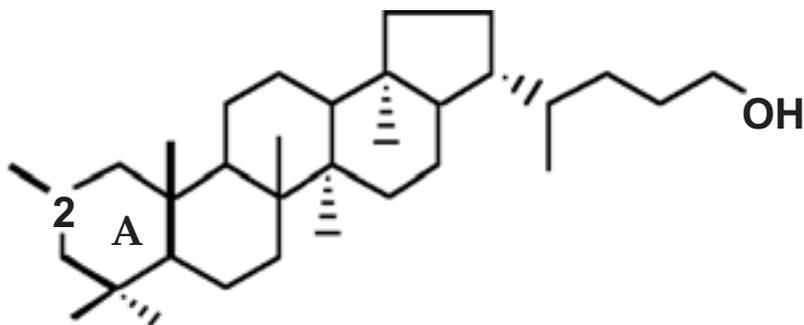


Figure 16. 2-Methylbacteriohopanol is one example of a class of amphiphilic membrane biochemicals which are a particularly important group of biomarker molecules.

therefore, only of general taxonomic use. A supplementary methyl substituent at the 2-position of ring-A of the pentacyclic triterpane skeleton, however, may provide some degree of biomarker specificity (see Figure 16).

Similar 2-methylhopanes have been identified in 2.5 billion year old organic rich shales by a member of our group (R.E. Summons, Australia Geological Survey Organization). Our culture studies of a taxonomically diverse spectrum of cyanobacteria has shown that methylation at C-2 is common. We have found that 2-methylbacteriohopanols (2-Me-BHP) are prominent membrane lipids in approximately 43% of cul-

tured cyanobacteria and a number of cyanobacteria-dominated environmental mat samples. Although not exclusive to this group of bacteria, the only other known sources of 2-Me-BHP occupy specialist environmental niches and do not appear capable of providing any quantitatively important contribution to sediments. 2-Me-BHP carry a readily measured molecular ^{13}C signature that will extend their usefulness as organism-specific biomarkers. Cyanobacteria are photoautotrophs using the energy of sunlight to convert carbon dioxide to cellular components, and as discussed above, such biological processes can result in carbon isotope fractionation. We have found that the BHP synthesized as a result of cyanobacterial, photoautotrophic growth are depleted in ^{13}C by 28 per mil relative to the carbon dioxide source with the 2-Me-BHP about 2 per mil heavier than its non-methylated analog. Similar carbon isotopic patterns have been measured for the 2-methyl and non-methyl BHPs in natural environmental samples establishing an isotopic signature for cyanobacterial BHP. Knowledge of the fractionation factors accompanying their biosynthesis also accord the potential to use 2-Me-BHP to determine the isotopic composition of inorganic carbon in modern aquatic and marine environments and their analogues in the fossil record. □

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Simulations of a Model Transmembrane Proton Transport System

Karl Schweighofer and Andrew Pohorille

The transport of protons across membranes is an essential process for both bioenergetics of modern cells and the origins of cellular life. All living systems make use of proton gradients across cell walls to convert environmental energy into a high-energy chemical compound, adenosine triphosphate (ATP). ATP, in turn, is used as a source of energy to drive many cellular reactions. The ubiquity of this process in biology suggests that even the earliest cellular systems were relying on proton gradient for harvesting environmental energy needed to support their survival and growth. In contemporary cells, proton transfer is assisted by large, complex proteins embedded in membranes. The issue addressed in this study was: how the same process can be accomplished with the aid of similar, but much simpler molecules that could have existed in the protobiological milieu?

The model system used in the study contained a bilayer membrane made of phospholipid, dimyristoylphosphatidylcholine (DMPC), which is a good model of the biological membranes forming cellular boundaries. Both sides of the bilayer were surrounded by water that simulated the environment inside and outside the cell. Embedded in the membrane was a fragment of the Influenza-A M₂ protein, and enough sodium counter ions to maintain system neutrality (see Figure 17).

This protein has been shown to exhibit remarkably high rates of proton transport and, therefore, is an excellent model to study the formation of proton gradients across membranes. The Influenza M₂ protein is 97 amino acids in length, but a fragment 25 amino acids long, which contains a transmembrane domain of 19 amino acids flanked by 3 amino acids on each side, is sufficient to transport protons. Four identical protein fragments, each folded into a helix, aggregate to form small channels spanning the membrane. Protons are conducted through a narrow pore in the middle of the channel, in response to an applied voltage. This channel is large enough to contain water molecules, and is normally filled with water.

In analogy to the mechanism of proton transfer in some other channels, it has been postulated that protons are translocated along the network of water molecules filling the pore of the channel. This mechanism, however, must involve an additional, important step because the channel contains four histidine amino acid residues, one from each of the helices, which are sufficiently large to occlude the pore and interrupt the water network. The histidine residues ensure channel selectivity by blocking transport of small ions, such as sodium or potassium. They have been also implicated in gating protons due to the ability of each histidine to become positively charged by accepting an additional proton. Two mechanisms of gating have been proposed.



In one mechanism, all four histidines acquire an additional proton and, due to repulsion between their positive charges, move away from one another, thus opening the channel. The alternative mechanism relies on the ability of protons to move between different atoms in a molecule (tautomerization). Thus, a proton is captured on one side of the gate while another proton is released from the opposite side, and the molecule returns to the initial state through tautomerization. The simulations were designed to test these two mechanisms.

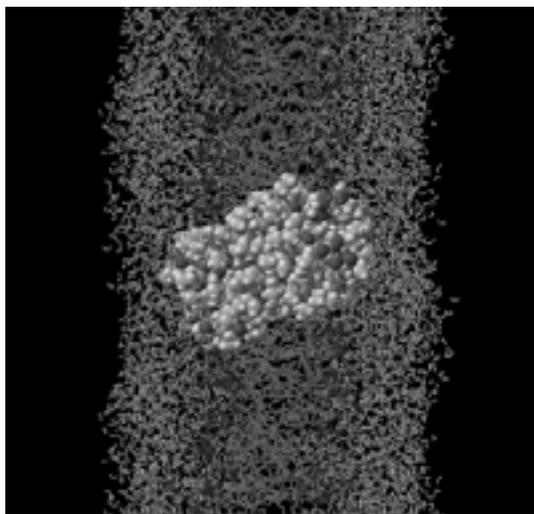


Figure 17. This figure shows the M_2 protein embedded in a DMPC membrane which is flanked by water layers. Both the water, and the membrane are represented by sticks, whereas the protein is rendered as spherical or 'cpk.' The color legend is as follows: water hydrogen (cyan), water oxygen (magenta), membrane hydrocarbon tails (green), membrane head groups (blue), sodium ions (yellow cpk), protein (multicolored cpk). The protein is clearly tilted in the bilayer, which helps it maintain favorable interactions with the interior of the membrane. Water in the channel cannot be seen because of the opacity of the protein in this figure.

Large-scale, atomic-level molecular dynamics simulations of the channel, with the histidine residues in different protonation states, revealed that all intermediate states of the system involved in the tautomerization mechanism are structurally stable and the arrangement of water molecules in the channel is conducive to the proton transport. In contrast, in the four-protonated state, postulated to exist in the gate-opening mechanism, the electrostatic repulsion between the histidine residues appears to be so large that the channel loses its structural integrity and one helix moves away from the remaining three. This result indicates that such a mechanism of proton transport is unlikely.

The simulations revealed that translocation along a network of water molecules in the channel and tautomerization of the histidine residues in the M_2 proteins is the most likely mechanism of proton transport. The results not only explain how a remarkably simple protein system can efficiently aid in the formation of proton gradients across cell walls, but also suggest how this system can be genetically re-engineered to become a directional, reversible proton pump. Such a pump can provide energy to laboratory-built models of simple cellular systems. If they were successfully constructed, it would greatly advance our understanding of the beginnings of life and find important applications in medicine and pharmacology. □

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Molecular Imprinting of Polymeric Columns

Thomas Shen, Jay Chen, and Narcinda Lerner

L-amino acids are one of the fundamental building blocks of living organisms. Because of this, their detection at trace levels is one of the major targets of many programs searching for the origin of life. L-amino acids have an advantage over other biomarkers in that they contain a chiral center, which is considered as a 'biosignature' for the presence of life. Amino acids of abiotic origin are racemic, with equal amounts of the L-form and the D-form. Any biological debris remaining from chemical and/or biological evolution on Mars or other planetary bodies would be present in trace amounts due to decay over millions or billions of years. Highly sensitive instruments will be required to detect such debris in situ as well as in measurements of returned samples from planetary flight missions.

Molecular imprinting is a simple and convenient way to prepare antibody-like materials which have been increasingly used in separation science and, more recently, sensing technology. The principle of this technique, including surface attachment and in situ polymerization, is described in Figure 18. Using this technology, very small amount of amino acids or biomolecules can be concentrated and separated for detection.

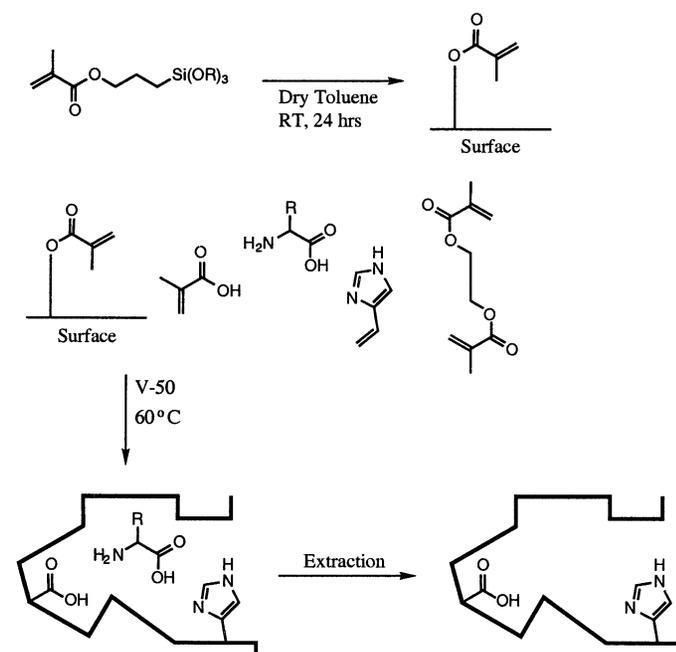


Figure 18. The preparation of surface-linked molecular imprinting polymeric columns.



Recently, the reaction conditions for preparing molecularly imprinted polymers (MIP) for amino acid separation were developed. The results indicated that the template effect exists by comparison of the binding effects between molecularly imprinted polymers and the control polymers. Approximately up to 1 microgram (μg) of L-phenylalanine (L-phe) could be absorbed by 1 milligram (mg) of L-phenylalanine-imprinted polymers.

Experiments on coating a capillary column with the L-phe-imprinted polymer by in situ polymerization were also studied. The inner surface of the capillary was first coated with a layer of the monomer trimethoxysilylpropyl methacrylate and then in situ polymerization was conducted inside the column (inner diameter = 0.53 millimeter) in the presence of L-phe.

Visual observation indicates that the capillary coated with the L-phe-imprinted polymer displays a nice layer of MIP coating on the inner surface of the column. The bonded polymer layer could be observed readily without instrumentation. Further analysis based on scanning microscopy will provide information regarding the bonding structure of this coating. The good structural stability of the stationary phase produced by this procedure will allow its use in the production of capillaries with reduced inner diameter i.e. micro-columns on glass chips. The success of coating the micro-columns with the MIPs will make possible miniaturized capillary electro-chromatography with a stationary phase designed specifically for targeted molecules.

Preliminary results indicate that small amounts of non-specific absorption also occurred, therefore, further improvement is in progress. □

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Prebiotic Synthesis of Activated Amino Acids

Arthur L. Weber

Chemical processes occurring on the primitive Earth about four billion years ago yielded molecules that had the ability to make copies of themselves (replicate). These rudimentary replicating molecules eventually developed into today's life that uses both protein and DNA molecules for replication. Since the DNA used by modern biota is too complex to have been chemically made on the primitive Earth, the first replicating systems may have been composed solely of small proteins ('peptides'). Peptides are good candidates for the first replicating molecules because they are constructed from very simple building blocks – activated amino acid molecules – which could have been made by chemical processes on the primitive Earth.

To determine how activated amino acid and peptide molecules could have been generated on, or delivered to the primitive Earth four billion years ago three distinct research areas were pursued: 1) the catalysis of sugar synthesis and its conversion to activated amino acids was investigated in the laboratory under simulated primitive Earth conditions, 2) the energy of carbon-carbon bond formation and cleavage for different carbon groups was calculated to evaluate the constraining role of thermodynamics in prebiotic carbon chemistry leading to the origin of life and metabolism, and 3) samples of the Cretaceous-Tertiary boundary layer were analyzed for extraterrestrial amino acids to see if they survived the meteorite impact that delivered them to

Earth. In the past year significant progress was made in these three research areas.

Amines (including amino acids) were shown to catalyze the prebiotic pathway (discovered last year) that generates activated amino acid thioesters capable of forming peptides. Specifically, amines were found to catalyze the first two reactions of the prebiotic pathway (aldol condensation and dehydration) involved in the synthesis of alanine and homoserine thioesters from formaldehyde and glycolaldehyde. Catalysis of the first two steps of this pathway by alanine, itself a product of the pathway, demonstrates that this prebiotic pathway has the potential to function autocatalytically. This pathway is an attractive prebiotic process because it operates under mild aqueous conditions, and like modern amino acid biosynthesis, uses sugar intermediates which are converted to amino acids by energy-yielding redox reactions.

The second line of investigation was to identify the thermodynamic constraints that govern carbon chemistry involved in the origin of life. The free energy was calculated for the formation and cleavage of 100 carbon-carbon bond combinations made from 10 different aliphatic carbon groups. Overall, this analysis showed that thermodynamics acts to constrain the carbon chemistry of the origin of life to reactions



that proceed by redox disproportionation. In disproportionation in which the more oxidized carbon group of a reacting pair of carbons becomes more oxidized, and the more reduced carbon group of the pair becomes more reduced. Furthermore, the analysis revealed three interesting characteristics of carbon bonds: a) carbon bonds to carbonyl groups are very unstable, making them easy to cleave but hard to make, b) alcohol groups are moderately unstable, making them more difficult to cleave but not hard to make, and c) hydrocarbon groups are very stable, making them very difficult to cleave but not hard to make.

In the final area of investigation, and in preparation for the analysis of the martian meteorite ALH84001, samples of Cretaceous-Tertiary boundary sediments were analyzed for extraterrestrial amino acids. A sample of Cretaceous-Tertiary boundary sediment from Sussex Wyoming was shown to contain about 310 picomoles per gram of the meteoritic amino acid, α -amino-isobutyric acid. Knowledge of the amino acids in extraterrestrial materials, such as meteorites, contributes to understanding the processes involved in the delivery of amino acids to the primitive Earth during the origin of life. □

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